CSIRO PUBLISHING

Marine and Freshwater Research
https://doi.org/10.1071/MF19163

Walking, swimming or hitching a ride? Phylogenetics and biogeography of the walking shark genus *Hemiscyllium*

Christine L. Dudgeon[A,H], *Shannon Corrigan*[B], *Lei Yang*[B], *Gerry R. Allen*[C], *Mark V. Erdmann*[D,E], *Fahmi*[A,F], *Hagi Y. Sugeha*[F], *William T. White*[G] and *Gavin J. P. Naylor*[B]

[A]The University of Queensland, School of Biomedical Sciences, Saint Lucia, Qld, 4072 Australia.
[B]Florida Museum of Natural History, Dickinson Hall, 1659 Museum Road, University of Florida, Gainesville, FL 32611, USA.
[C]Department of Aquatic Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Perth, WA 6986, Australia.
[D]Conservation International Indonesia Marine Program, Jalan Dr Muwardi 17, Renon, Denpasar 80235, Indonesia.
[E]California Academy of Sciences, Golden Gate Park, San Francisco, CA 94118, USA.
[F]Research Centre for Oceanography, Indonesian Institute of Sciences (P2O LIPI), Jalan Pasir Putih 1, Ancol Timur, Jakarta 14430, Indonesia.
[G]CSIRO Australian National Fish Collection, National Research Collections Australia, GPO Box 1538, Hobart, Tas. 7000, Australia.
[H]Corresponding author. Email: c.dudgeon@uq.edu.au

Abstract. It can be challenging to identify the forces that drive speciation in marine environments for organisms that are capable of widespread dispersal because their contemporary distributions often belie the historical processes that were responsible for their initial diversification. In this contribution we explore the likely sequence of events responsible for the radiation of walking sharks in the genus *Hemiscyllium* using a dated molecular phylogeny. The nine currently recognised species in the genus consist of small, benthic sharks that are restricted to the Indo-Australian Archipelago and show limited dispersal at both juvenile and adult stages. We discuss how major tectonic changes, sea level fluctuations and the unique biology of the species may have influenced speciation in the group, as well as the current distribution of the genus and each of its constituent species. Phylogeographic analysis of the genus combined with biogeographic reconstruction of the region shows a recent radiation during the Miocene and Pliocene, and supports a combination of vicariance and founder modes of speciation mediated by major tectonic, geological and oceanographic historical processes.

Additional keywords: Australia, eastern Indonesia, epaulette shark, New Guinea, radiation, Sahul region.

Received 7 May 2019, accepted 28 October 2019, published online 21 January 2020

Introduction

Geographic processes can play a major role in generating biodiversity by promoting reproductive isolation of taxa separated by barriers. However, identifying the causal agents behind speciation processes is challenging, because contemporary distributions and patterns of genetic diversity often mask historical signatures (Barraclough and Nee 2001). This is exacerbated in the marine realm where there are few impermeable barriers and the dispersal potential of most organisms is high, either as pelagic larvae for many benthic invertebrate and teleost species (Barber 2009) or as adults in pelagic teleosts and elasmobranchs (Heist 2004). For much of the past century, allopatric speciation was favoured as the prevalent mode of speciation (Turelli *et al.* 2001; Coyne and Orr 2004; Skeels and Cardillo 2019). However, there is increasing support for other modes, including founder (establishment of new populations and species through long-distance dispersal), parapatric and sympatric (Turelli *et al.* 2001; Nosil *et al.* 2009) speciation. In a recent meta-analysis by Skeels and Cardillo (2019), phylogenetic signals in most of the animal groups examined, including the coral reef fish genus *Amphiprion* as an example from the marine environment, were consistent with founder speciation processes rather than allopatric speciation.

Few marine organisms have restricted ranges with limited dispersal capacity at both juvenile and adult stages. One example is the group of species within the small benthic shark genus

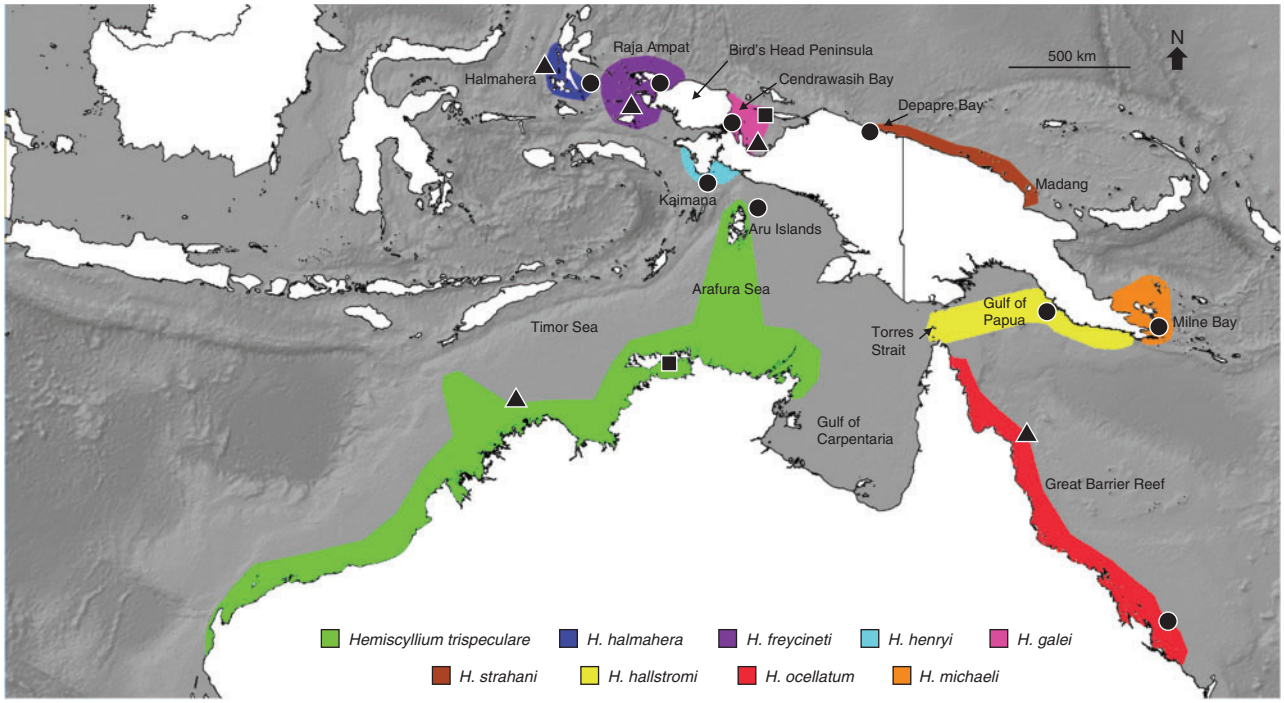

Fig. 1. Map showing the Island of New Guinea, northern Australia and islands of eastern Indonesia. The shading corresponds to the distributions of the nine species of *Hemiscyllium* (following Allen *et al.* 2016). The black shapes represent the sampling sites and correspond to the specific samples shown in Fig. 3 and Table 1.

Hemiscyllium Müller & Henle 1838. Known as walking or epaulette sharks, species within *Hemiscyllium* are small (to 107-cm total length, but mostly <70 cm), nocturnally active benthic sharks associated with shallow reef and seagrass habitats (Allen *et al.* 2016; Weigmann 2016). Knowledge of the biology of the genus is largely restricted to *Hemiscyllium ocellatum*. This species demonstrates several adaptive traits to living in shallow, restricted environments with limited dispersal potential, including a physiological adaptation to coping with hypoxic conditions during low tide periods (Wise *et al.* 1998). *H. ocellatum* (Compagno 2001) and *Hemiscyllium trispeculare* (W. T. White, pers. obs.) are oviparous and juveniles hatch out as essentially independent miniature versions of the adult sharks. Given all members of the other genus (*Chiloscyllium* Müller & Henle 1837) within the family Hemiscylliidae for which reproduction is known are also oviparous (Compagno 2001; Weigmann 2016), it is likely that this is the reproductive mode for all hemiscylliids. All *Hemiscyllium* species share a unique form of locomotion, wherein they use their highly muscular paired fins to essentially 'walk' along the substrate while foraging for benthic invertebrates and fishes (Allen *et al.* 2016).

The genus *Hemiscyllium* is endemic to the shallow coastal environments in the Sahul region (continent of Australia, New Guinea and surrounding islands) of the Indo–Australian Archipelago. The species are distributed around the island of New Guinea and within the tropical waters of northern Australia, as well as satellite islands to the west of New Guinea including the Raja Ampat Archipelago, Halmahera and Aru (Allen *et al.* 2016; Fig. 1). The western-most boundary broadly coincides with Weber's Line, which represents the split between a prominently Indo–Malayan terrestrial fauna to its west and a Papuan fauna to its east (Mayr 1944). Earlier reviews by Dingerkus and DeFino (1983) and Compagno (2001) recognised five species of *Hemiscyllium: H. freycineti* Quoy & Gaimard 1824–25, *H. hallstromi* Whitley 1967, *H. ocellatum* Bonnaterre 1788, *H. strahani* Whitley 1967 and *H. trispeculare* Richardson 1843. Four additional *Hemiscyllium* species have been described during the past 11 years: *H. galei* Allen & Erdmann 2008, *H. henryi* Allen & Erdmann 2008, *H. michaeli* Allen & Dudgeon 2010 and *H. halmahera* Allen, Erdmann & Dudgeon 2013. A recent review of the genus *Hemiscyllium* provides updated distributions and descriptions for all nine species (Allen *et al.* 2016). All species are similar in body size and morphology, but can be readily differentiated based on colour patterns (Allen *et al.* 2016). Prior to the recent descriptions, the five recognised species were thought to have substantial overlap in their distributions. In particular, the previously reported distribution for *H. ocellatum* extended throughout most of the range of the genus (Compagno *et al.* 2005). Conversely, the present nine recognised species exhibit adjacent, disjunct distributions (Fig. 1). Similar non-overlapping species distributions have been noted for other recently diverged species and have largely been attributed to vicariant processes (Barraclough and Nee 2001).

The seas surrounding the island of New Guinea and northern Australia overlap 'The Coral Triangle' marine biodiversity hot spot (Allen and Erdmann 2008) and are among the most biodiverse on the planet. The hot spot has its origins in

Table 1. Summary information for sample numbers and sampling locations for the different *Hemiscyllium* species
The number of haplotypes per location and in total, species nucleotide diversity and mean *P*-distances with standard errors are presented for the NADH dehydrogenase subunit 4 (*NADH4*) sequence data

Species	Sampling location	Number of samples	Number of haplotypes	Total number of haplotypes	Nucleotide diversity	Mean (±s.e.m.) *P*-distances
Hemiscyllium freycineti	Kri Island, Raja Ampat, Indonesia	5	2	2	0.0013	0.001 ± 0.001
	Aljui Bay, Raja Ampat, Indonesia	1	1			
Hemiscyllium galei	Rumperpon Island, Cenderawasih Bay, Indonesia	3	1	1	0	0.000 ± 0.000
	Kwatisore, Cenderawasih Bay, Indonesia	4	1			
	North Yapen, Cenderawasih Bay, Indonesia	1	1			
Hemiscyllium hallstromi	Lion Island, Bootless Bay, Papua New Guinea	3	1	1	0	0.000 ± 0.000
	Loloata Island, Bootless Bay, Papua New Guinea	4	1			
Hemiscyllium halmahera	Ternate, Halmahera, Indonesia	2	2	3	0.0071	0.007 ± 0.002
	Weda Bay, Halmahera, Indonesia	2	1			
Hemiscyllium henryi	Selat Iris, Kaimana, Indonesia	1	1	1	0	0.000 ± 0.000
	Triton Bay, Kaimana, Indonesia	3	1			
Hemiscyllium michaeli	Nuakata, Milne Bay, Papua New Guinea	6	1	1	0	0.000 ± 0.000
Hemiscyllium ocellatum	Heron Island, Australia	5	2	3	0.0021	0.002 ± 0.001
	Lizard Island, Australia	1	1			
	Cairns, Australia	1	1			
Hemiscyllium strahani	Depapre Bay, Jayapura, Indonesia	6	1	1	0	0.000 ± 0.000
Hemiscyllium trispeculare	Taberfane, Aru Islands, Indonesia	3	1	4	0.0053	0.005 ± 0.002
	Channel Island, Darwin, Australia	2	2			
	K/09 Adele Island, Kimberley, Australia	1	1			
	K/10 Cassini Island, Kimberley, Australia	1	1			

the Miocene epoch (Renema *et al.* 2008). The mid-Miocene (*c.* 10 Ma) coincided with the completion of the northward movement of Australia and New Guinea, and the increase of land mass of northern New Guinea due to extensive volcanism (Hall 1998). The collision of the Australian plate with the Pacific Arcs and South-east Asian margin during this period resulted in the creation of new island and shallow sea habitat, likely leading to this increased marine biodiversity (Renema *et al.* 2008).

The aim of this study was to conduct a comprehensive molecular phylogenetic analysis of the genus *Hemiscyllium* based on whole mitochondrial genome sequences of a complete taxonomic sampling of all nine currently recognised species. Furthermore, multiple representatives per species were examined with the NADH dehydrogenase subunit 4 (*NADH4*) marker to provide insight into intraspecific variation and potential population structuring. Due to limited dispersal capacity at both juvenile and adult stages, and the generally restricted distributions of most species, the phylogenetic patterns are likely to be representative of historical processes leading to diversity in this group as well as contemporary patterns. We discuss the results with respect to the tectonic and hydrological history of the region and the unique biology of the genus *Hemiscyllium* to inform speciation processes and generation of biodiversity in this genus.

Materials and methods

Taxon sampling

Tissue samples were collected from live-caught specimens or from verified specimens in research or museum collections (Table 1; see also Table S1, available as Supplementary material to this paper). Live specimens were caught by hand and either a small piece of tissue removed from a fin and the animal released or the animal retained for museum collections (see Allen and Erdmann 2008). All sharks collected for specimens were killed by submerging in a 25% clove oil/75% ethanol anaesthetic solution for 15 min according to Conservation International–Indonesia's ethics policy for marine fish collection. Tissue was preserved in 80–100% ethanol for downstream molecular analysis. DNA was extracted using either the Isolate II DNA Extraction (Bioline, Sydney, NSW, Australia) or the EZNA Tissue DNA (Omega Bio-Tek, Norcross, GA, USA) kit according to the manufacturers' instructions.

Data generation: library preparation, DNA hybridisation capture and sequencing

Genomic DNA was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). A single representative of each species was subjected to targeted DNA hybridisation capture for the purposes of rapidly collecting complete mitochondrial genome sequences. Genomic DNA (0.5–3 μg per sample) was sheared to ~500 bp using acoustic ultrasonication on a Covaris M220 Focused-ultrasonicator (Covaris, Woburn, MA, USA). Illumina (San Diego, CA USA) sequencing libraries (Meyer and Kircher 2010) were then prepared using the 'with-bead' method (Fisher *et al.* 2011), following Li *et al.* (2013). A custom biotinylated RNA bait library based on sequences derived from 99 shark species (MYbaits MYcroarray, Ann Arbor, MI, USA) was used to target the entire mitochondrial genome in an individual cross-species DNA hybridisation capture experiment per taxon, following the relaxed hybridisation method described by Li *et al.* (2013). Enriched libraries were pooled in equimolar ratios and pooled libraries were diluted to 12–15 pM for paired-end 250- to 300-bp sequencing on an Illumina MiSeq benchtop sequencer (Illumina). Sequence reads associated with each sample were identified and sorted by their respective indices (Li *et al.* 2013). Adapters and low-quality reads were removed using Cutadapt and FastQC available in the wrapper script Trim Galore! (ver. 0.3.1, F. Krueger, see https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/, accessed 1 July 2015). Trimmed mitochondrial sequence reads were imported into Geneious Pro (ver. 7.1.9, Biomatters, Auckland, New Zealand; and unique reads were retained and mapped to a reference sequence of *Chiloscyllium plagiosum* (sequenced by L. Yang).

The mitochondrial *NADH4* region was also sequenced for multiple individuals of each species (678 bp for 53 individuals), as described in Allen and Erdmann (2008). This was conducted in order to explore intra- *v.* interspecific divergences for larger sample sizes than could be included in our DNA hybridisation capture experiment. The *NADH4* marker was chosen to build on previous studies that had demonstrated its effectiveness at discriminating between species and population levels within the genus (Allen and Erdmann 2008; Allen and Dudgeon 2010; Allen *et al.* 2013).

Phylogenetic analysis

Two datasets were compiled; one included the expanded sampling of *NADH4* sequences (referred to as the '*NADH4* dataset') and the other included the protein-coding components (for ease of alignment) of the whole mitochondrial genome sequences (referred to as the 'mitogenome dataset') for a representative of each species. The sequences for each dataset were aligned across all taxa plus one representative of *Chiloscyllium punctatum* (as an outgroup) using Geneious Pro (ver. 7.1.9, Biomatters). This yielded alignments that were 678 and 11 430 bp in length, including 76 and 494 parsimony-informative sites for the *NADH4* and mitogenome datasets respectively. In the mitogenome dataset, the complementary strand sequences were used for NADH6, which is encoded on the L-strand. Alignment quality was confirmed by translation of both datasets and incomplete stop codons were excluded. Representative *NADH4* sequences are available through GenBank (Accession numbers MF740829–MF740846).

Phylogenetic analyses were performed in PAUP* (ver. 4.0a148, see https://paup.phylosolutions.com/, accessed 1 August 2016; Swofford 2002), and internal model test options were used to select the model of sequence evolution for each dataset based on Akaike's information criterion corrected for small sample size (AICc) ranking. The maximum likelihood (ML) tree was estimated for the *NADH4* dataset using a heuristic search and the Tamura–Nei (TrN) + gamma distribution (G; $\alpha = 0.31$) model of sequence evolution. The ML tree for the mitogenome dataset was estimated using a heuristic search and the general time reversible (GTR) + proportion of invariable sites (I) + G ($\alpha = 0.72$, pinvar = 0.49) model of sequence evolution. Nodal support was estimated in both cases by performing 100 bootstrap replicates.

Divergence time estimation

Divergence time was estimated using BEAST (ver. 1.8.3, see http://beast.community/beast/, accessed 20 April 2019; Drummond and Rambaut 2007; Drummond *et al.* 2012) by the CIPRES Science Gateway (ver. 3.3, see http://www.phylo.org/, accessed 20 April 2019; Miller *et al.* 2010). For calibration purposes, we added one more species (*Rhincodon typus*) to the mitochondrial genome dataset. The dataset was partitioned according to codon positions and the GTR+I+G model was used. The stem node of Hemiscylliidae, with a hard lower bound and a soft upper bound, was used for calibration. The lower bound (93.9 Ma) was based on the oldest known hemiscylliid fossil, the extinct *Mesiteia emiliae* from the Cenomanian of the Late Cretaceous (Cappetta 1980), whereas the upper bound (100.5 Ma) was based on the oldest known orectolobiform fossil from the Upper Albian of the Early Cretaceous (Siverson 1997). Input parameters included a Log(Stdev) set to 0.5 to reflect the degree of confidence in the fossil record. The upper bound was sampled following a log-normal distribution after setting the offset (lower bound) as 93.9 and the Log(Stdev) as 0.5. The Log(Mean) value was set to 1.058 to constrain the upper bound value to be 100.5 at 95% of the distribution. Two independent runs (500 million generations each; sampling frequency = 1000) were performed. An uncorrelated log-normal relaxed clock model was used. The birth–death process was chosen for the prior (an input parameter) to inform the tree generation. Results from both runs (burn-in = 100 million generations for each run) were combined using LogCombiner (ver. 1.1.0, A. Rambaut and A. J. Drummond, see http://beast.community/logcombiner/, accessed 20 April 2019). The maximum clade credibility (MCC) tree was created and the 95% highest posterior density (HPD) limits of the node heights were summarised. Phylogenetic trees were visualised using FigTree (ver. 1.4.2, see http://tree.bio.ed.ac.uk/software/figtree, accessed 10 February 2017) and edited in Adobe Illustrator CC (ver. 2015.2.0, Adobe, San Jose, CA, USA).

Results

Whole mitochondrial genome phylogeny and divergence time estimates

Interspecific pairwise sequence divergence (uncorrected p-distance) based on the mitochondrial genome dataset

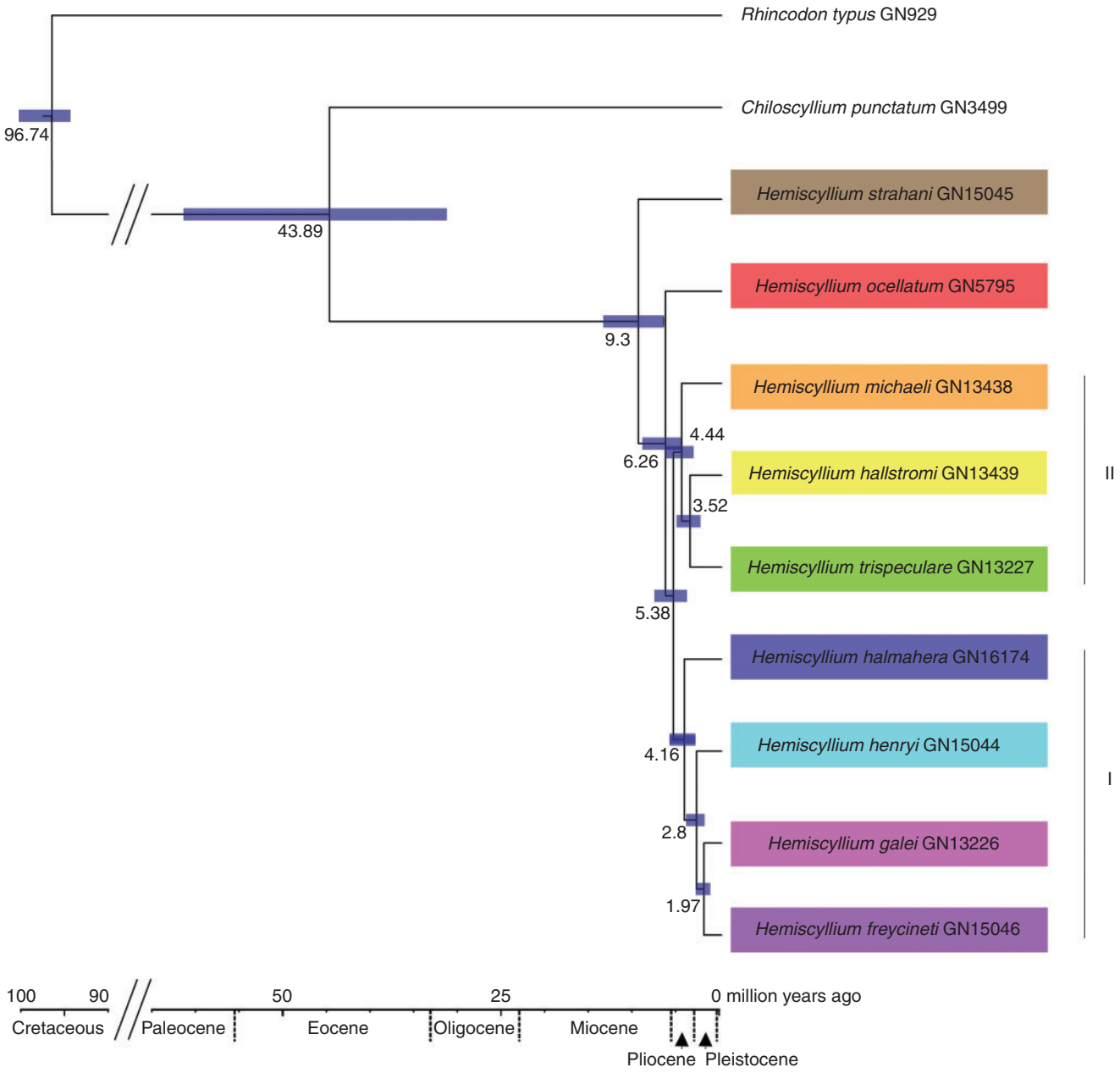

Fig. 2. Time-calibrated maximum likelihood tree for the mitogenome sequence data for alignment of the protein coding components of the mitochondrial genomes (11 430 sites) for *Hemiscyllium.* Bars indicate 95% highest posterior densities of divergence dates with means estimated in million years ago given at the nodes. Bayesian posterior probability values were 1 for all nodes except for the split between *Chiloscyllium* and *Hemiscyllium*, which had a value of 0.994. The two terminal clades are shown: Clade I and Clade II.

ranged from 0.010 (*H. galei v. H. freycineti*) to 0.040 (*H. strahani v. H. freycineti* and *H. henryi*), with an average of 0.025.

The ML tree inferred from the whole mitochondrial genome dataset is well resolved with high bootstrap support (>93%) for all major nodes (Fig. S1). The northern Papuan species *H. strahani* and the Australian species *H. ocellatum* are basal to a clade containing the two major reciprocally monophyletic groups. The first group encompasses Bird's Head Peninsula endemics *H. halmahera*, *H. freycineti*, *H. galei* and *H. henryi*; the second includes the northern Australian–Arafura Sea species *H. trispeculare* and the south-eastern Papuan species *H. michaeli* and *H. hallstromi*. The diversification time analyses indicated the split between *Hemiscyllium* and *Chiloscyllium* at *c.* 44.82 Ma (Eocene; 95% HPD: 31.44–61.54 Ma). However, *Hemiscyllium* is not estimated to diversify until *c.* 9.5 Ma (mid- to late Miocene; 95% HPD: 6.58–13.46 Ma) and most diversification within this genus seems to have occurred through the late Miocene and Pliocene (Fig. 2).

NADH4 *phylogeny*

The topology of the ML tree based on analysis of the *NADH4* dataset was largely consistent with that inferred from the whole mitogenome data. Species clustered similarly into Bird's Head Peninsula and Australian–Arafura clades, although *H. freycineti* was inferred to be paraphyletic in the *NADH4* analysis. *H. ocellatum* was inferred to be basal in the *NADH4* analysis, rather than *H. strahani*. However, most nodes in the *NADH4* phylogeny were not strongly supported (Fig. 3), indicating a lack of signal in this dataset for inferring species interrelationships. Although the *NADH4* data provided limited signal for inferring species interrelationships, species groups were resolved with strong bootstrap support and showed intraspecific variation for four of the nine species, potentially indicating population structure. Uncorrected intraspecific p-distance based on the *NADH4* data ranges between 0 and 0.010, with an average of 0.002. For the northern Australian species *H. ocellatum* and *H. trispeculare*, which have the widest geographic distributions within the genus, variation was revealed across wide geographic scales spanning hundreds of kilometres (Fig. 1, 3). Intraspecific variation was evident over tens of kilometres for *H. halmahera* and *H. freycineti*. For *H. halmahera*, the sampling sites span either side of the main island mass, whereas in *H. freycineti* there is no contemporary major geographic barrier to dispersal. In contrast, *H. galei* was sampled from three locations spanning its current distribution across a similar geographic scale to *H. freycineti* with no evidence of population structuring. For the other four species, sampling occurred only at one location and no intraspecific variation could be determined.

Discussion

The results of this study are consistent with a recent species radiation in the genus *Hemiscyllium*, commencing in the Miocene, within the Indo–Australian Archipelago associated with widespread creation of suitable shallow-water carbonate habitat (Hall 1998; Renema *et al.* 2008). *Hemiscyllium* is recorded from the Late Cretaceous period through to the Eocene epoch from fossil deposits of teeth in Europe (Belgium, Germany, Denmark, Sweden, UK, France) North and Central America, North Africa and India (Pollerspöck and Beaury 2014). Our diversification time estimates, based on mitogenome data, date the split between *Hemiscyllium* and *Chiloscyllium* broadly coinciding with these periods, centred during the early Eocene (*c.* 45 Ma). This lends support to a Tethyan origin for the genus. There is a lack of fossil records for *Hemiscyllium* following the Eocene until the Pleistocene (Pollerspöck and Beaury 2014), supporting a recent species radiation. This may be due to few surviving lineages following Cretaceous–Tertiary boundary mass extinction events. This period was characterised by increasing sea levels followed by drops in ocean temperatures, along with meteorite strikes and volcanic activity (Adolfssen and Ward 2014). Fossil deposits demonstrate a reduction in the number of neoselachian genera, with demersal shallow marine forms considered to be heavily affected (Kriwet and Benton 2004). The carpet shark order Orectolobiformes, which includes the Hemiscylliidae, is largely composed of shallow benthic and demersal forms (with the exception of the whale shark *R. typus*) and is considered be one of the elasmobranch groups most affected by mass extinctions during this time (Pollerspöck and Beaury 2014).

The mitochondrial genome phylogeny indicates that *H. strahani* is the most basal taxon, having diversified in the mid-Miocene. This contrasts markedly with Dingerkus and DeFino (1983), who concluded that *H. strahani* was the most derived of the genus based on its distinctive adult colour pattern and *H. ocellatum* was the most basal taxon because it had the most similar patternings between juvenile and adult forms of all *Hemiscyllium*. The mitogenome phylogeny also supports an older split for the speciation of *H. ocellatum*, with the lineage arising in the late Miocene, followed by a species radiation into two major clades. Although contemporary distributions may not represent historical origins, because *Hemiscyllium* is adapted to tropical waters it would likely have been restricted to the most northern parts of the Sahul region, extending further south while the region moved north into warmer waters.

The four species in Clade I (Fig. 2) occupy non-overlapping ranges that ring the Bird's Head Peninsula of north-west New Guinea and extend to the satellite island of Halmahera (Fig. 1). *Hemiscyllium* may have reached this region by ancestral migration from the south-east or north-east of New Guinea. However, *H. halmahera*, the most westerly distributed of the four species, is also the most basal within the clade. This distribution is consistent with colonisation of the Bird's Head Peninsula out of the west from the Halmahera Islands, but other scenarios are also possible. Allen *et al.* (2013) proposed that the colonisation of Halmahera by *Hemiscyllium* occurred when it was positioned further east as part of the Philippine–Halmahera arc system. During the early to mid-Miocene, the Philippine–Halmahera Sea Arc came into contact with northern New Guinea as the Australian plate migrated northward. Later during the Oligocene, Halmahera and surrounding islands, as well as parts of present-day northern New Guinea, formed a broadly continuous arc system (de Jong 1998). The subsequent strike-slip movements associated with the Sorong fault system (Hall 2002) shifted the Halmahera Archipelago westward to its present position off the Bird's Head Peninsula between 3 and 1 Ma (Hill and Hall 2003; Fig. 4). This could be considered a form of founder speciation through dispersal whereby the shark has 'hitched a ride' with the migrating islands. Similar patterns of dispersal by island-arc fragmentation have been proposed for New Guinea freshwater *Heteroptera* (water striders; Polhemus and Polhemus 1998). Such dispersal mechanisms may also be responsible for the presence on Halmahera of fish species that are otherwise restricted to the island of New Guinea, including the pseudochromids *Pseudochromis ammeri* Gill, Allen and Erdmann 2012, *Pseudochromis matahari* Gill, Erdmann and Allen 2009, and *Pseudochromis pylei* Randall and McCosker 1989, the nemipterid *Pentapodus numberii* Allen and Erdmann 2009 and the blenniid *Ecsenius randalli* Springer 1991. Furthermore, a single species of bird-of-paradise *Semioptera wallacii* (Gray, 1859) occurs on Halmahera, whereas the rest of the family Paradisaeidae is otherwise considered endemic to the island of New Guinea and Australia (Irestedt *et al.* 2009).

Between 10 and 2 Ma, many terranes started docking in the Bird's Head Peninsula (Pandolfi 1993), which may have facilitated speciation both through the generation of new habitat and creating barriers to dispersal (Fig. 4). From *c.* 10 to 4 Ma, there

Fig. 3. Phylogenetic tree showing the relationships among *Hemiscyllium* species, relative to the outgroup *Chiloscyllium punctatum*. The tree was derived from a maximum likelihood (ML) analysis of an alignment of the NADH dehydrogenase subunit 4 (*NADH4*) region of the mitochondrial genome (678 sites). Numbers on the nodes represent the ML bootstrap values. Black symbols relate to the sampling sites within the corresponding species distribution as shown in Fig. 1.

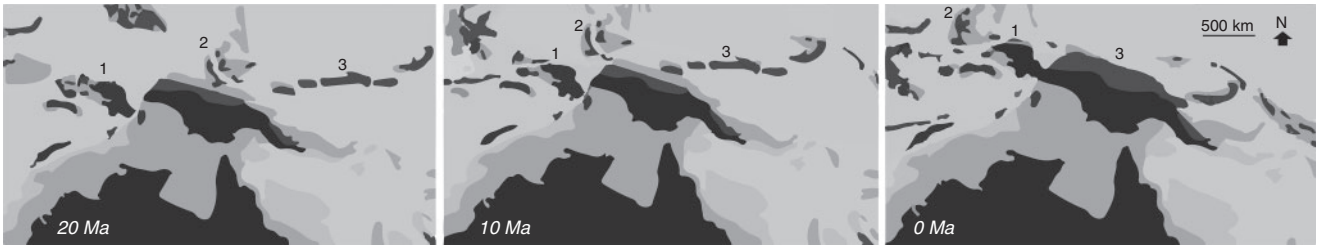

Fig. 4. Reconstruction of northern Australian, New Guinea and East Indonesia between 20 Ma and the present day. Reproduced with permission from Hall (2002). The geographic positions of three major features are indicated: (1) the Bird's Head Peninsula, (2) the Halmahera islands; and (3) the terranes prior to and after docking along northern New Guinea. Black, continental land mass; dark grey, arcs, ophiolitic and accreted material; medium grey, submarine parts of the coastal margins; light grey, oceanic region.

were extensive shallow sea areas and possible land surrounding the Bird's Head region (Hall 2009), potentially enabling easterly migration of *Hemiscyllium* from Halmahera. In the Late Pliocene, the region subsided again and has remained submerged, with the exception of the island of Misool. The terranes accreted in this region during the Late Pliocene, across the northern margin of the Bird's Head Peninsula and within Cenderawasih Bay (Pigram and Davies 1987; Pandolfi 1993), may be relevant to the split of *H. galei* and *H. freycineti*, which we estimate to be between 1.27 and 2.95 Ma. Current records separate these species by only ~150 km. *H. galei* is endemic to Cenderawasih Bay, whereas *H. freycineti* is more widely distributed across the Raja Ampat Archipelago (including south to Misool and eastwards to Amsterdam Island off the northern Bird's Head Peninsula). Contemporary separation may be maintained by riverine run-off, as well as a distinct lack of reef habitat across the top of the Bird's Head due to persistent exposure to high wave energy. Riverine outflows and extensive mangrove swamp habitat likely restrict the distribution of this clade easterly on both the northern and southern parts of the Bird's Head Peninsula (Allen *et al.* 2016).

The second clade (Fig. 2, Clade II) comprises three species and includes the Papua New Guinean endemics *H. michaeli* and *H. hallstromi*. The third species, *H. trispeculare*, is the most widely distributed within *Hemiscyllium*, found through western and northern Australian waters and the Aru Archipelago. Of these three species, *H. michaeli* split off first, consistent with an eastern origin for the group with subsequent southerly and westerly movement. The extent of the distributions for *H. michaeli* and *H. hallstromi* in south-eastern PNG are unknown and there appears to be potential for range overlap along the south-eastern cape between Orangerie and Milne bays. However, we found no support for this in the present study because the species are clearly divergent and no haplotype sharing was observed between species within the *NADH4* dataset. However, it should be noted that this study only focuses on the mitochondrial genome, and further sampling and investigation of nuclear markers may reveal different patterns.

The Torres Strait land bridge is likely to have played a role in the speciation of both *H. hallstromi* and *H. trispeculare*. *Hemiscyllium* may have colonised north-west Australia during the Miocene when the northern epicontinental sea that spanned between continental Australia and the New Guinean terranes would have facilitated movement (Wilson 2013). Sedimentation during the Pliocene period resulted in the separation of the Arafura and Coral seas, effectively acting as a vicariant barrier to marine dispersal, interspersed with periods of high sea levels opening the Torres Strait (Wilson 2013). An interesting point of potential secondary contact, which requires further investigation, is the presence of both *H. ocellatum* and *H. hallstromi* in the Torres Strait. Based on body patterning, these two species are more similar to each other than to other *Hemiscyllium* (Allen *et al.* 2016), but they do not appear to constitute sister species based on the current phylogenetic analysis. The contemporary geographic proximity of these two species within the Torres Strait may indicate post-speciation secondary contact. Representatives from the Torres Strait were not included in this study, but clearly this is a region of particular interest that should be the focus of future investigations.

The physiological adaptation of *Hemiscyllium* to low oxygen environments, as well as shallow water habitat preferences, may underlie current species distribution patterns and explain the lack of overlapping species ranges around New Guinea. Although *Hemiscyllium* are thought to have low dispersal potential associated with habitat specificity, their capacity for migration given appropriate contiguous habitat is demonstrated by the broad-scale distributions of the two Australian species *H. ocellatum* and *H. trispeculare*. *H. ocellatum* is distributed from the tip of Cape York to the southern extent of the Great Barrier Reef in the Tropic of Capricorn, although an individual record is reported further south from Sydney (Last and Stevens 2009). The extent of population subdivision within its range is unknown, but intraspecific variation is evident based on our expanded sampling of *NADH4* data, with population structure between the single sample from Lizard Island and those from Heron Island, separated by ~1200 km (Fig. 3). Future sampling efforts along the full extent of the range of *H. ocellatum* should further elucidate the extent of genetic connectivity between these populations.

H. trispeculare is the most widespread species in the genus, distributed from Coral Bay in Western Australia to the Northern Territory and north to the Aru Archipelago of eastern Indonesia, a relic of the former land bridge connecting Australia and New Guinea (Allen *et al.* 2015) situated ~110 km south of the West Papuan mainland. *H. trispeculare* is also reported from Ashmore Reef, which lies 350 km off the Kimberley Coast of Western Australia, although still connected to the continental shelf. Population subdivision is evident between the sampling

locations of Darwin, the Kimberley Coast of Western Australia and the Aru Islands based on the *NADH4* phylogeny. Similar to the east Australian coast, much of this region has been exposed repeatedly during low water level periods during the Pleistocene glaciation events. During the last glacial maximum, *c.* 17 000 years before present, sea levels were 120 m below current levels and remained as low as 50 m below current sea levels until *c.* 11 000 years before present (Voris 2000; Yokoyama *et al.* 2001). The contemporary coastal shelf and coral reef habitat have only developed within the past 10 000 years (Carter and Johnson 1986; Webster *et al.* 2018). Current population structure along these coastlines could be indicative of recolonisation after Pleistocene glaciation from offshore Pleistocene refugia, as has been proposed for other marine species (Wörheide *et al.* 2002; Lukoschek *et al.* 2007; van Oppen *et al.* 2011; Rosser 2016). A particular point of interest is the comparatively small geographic distance spanning ~200 km between Aru and Triton Bay, where *H. trispeculare* and *H. henryi* occur respectively. The lack of geographic overlap and divergent positioning of *H. trispeculare* and *H. henryi* in different clades on the phylogenetic trees lend support to the western origin of Clade I and the eastern origin of Clade II, as well as deep water providing an effective barrier to migration. There are currently no records of *Hemiscyllium* from the coastline of southern New Guinea spanning from Triton Bay in the west in an eastward direction to the Torres Strait. This may be due to the lack of suitable habitat, because this coastline has extensive riverine run-off, mangrove forests, mud plumes and substrates that are not typically associated with *Hemiscyllium*. However, comprehensive surveys are lacking for this region (except for trawl surveys by the Environmental Department of Freeport Mining Co.; K. Hortle, pers. comm.).

Conclusion

Hemiscyllium provides an interesting case study to examine speciation in the Indo–Australian Archipelago for an organism with limited dispersal at all life stages. Phylogenetic analysis of the genus combined with biogeographic reconstruction of the region shows a recent radiation of the nine species with adjacent endemic distributions suggesting a combination of vicariance and founder modes of speciation. Barriers may have arisen through movement of land masses and the creation of new land masses, including the ongoing accretion of terranes along the northern and western coastlines of New Guinea (Polhemus and Polhemus 1998; Hill and Hall 2003). Eustatic sea level fluctuations are likely to have played a role in reproductive isolation, in particular separating the western and eastern Australian coastlines (Wilson 2013). Although *Hemiscyllium* demonstrates limited dispersal, there is also evidence for founder speciation through direct dispersal into newly created suitable habitat and indirect dispersal: *Hemiscyllium* may have 'hitched a ride' with the Halmahera island group into eastern Indonesia during periods of strike-slip tectonic movements (Wilson 2013). Future research should focus on expanding sampling efforts across the distributions of each species, particularly the more widespread species such as *H. ocellatum*, *H. trispeculare*, *H. hallstromi* and *H. strahani*. Particular areas of interest also include potential zones of overlap for species such as the Torres Strait and the eastern tip of Papua New Guinea (Milne Bay Province), the northern Bird's Head Peninsula between Amsterdam Island and Manokwari, the north Papuan coastline between Jayapura and Yapen Island and the Huon Gulf region between Lae and Tufi in south-eastern Papua New Guinea. Further investigation in these areas may reveal contemporary processes of genetic segregation as well as potential hybridisation.

Conflicts of interest

C. L. Dudgeon is an Associate Editor with *Marine and Freshwater Research*; however, this was not the case while this paper was being prepared. As such, she did not at any stage have editorial-level access to the manuscript while it was in peer review. *Marine and Freshwater Research* encourages its editors to publish in the journal and they are kept totally separate from the decision-making processes for their manuscripts. The authors have no further conflicts of interest to declare.

Declaration of funding

The authors gratefully acknowledge the following institutions and individuals for their financial support of the fieldwork that collected the majority of samples analysed: Conservation International, the Paine Family Trust, the Walton Family Foundation, the Henry Foundation and Mickey Rosenau and Ellen Gritz. Financial support for the phylogenetic analyses was provided by National Science Foundation Division of Environmental Biology 1036500.

Acknowledgements

The authors extend their appreciation to Michael Hammer, Dion Webb, Clint Chapman and Sue Morrison for providing additional samples for this study. The authors also thank the Indonesian Department of Nature Conservation (PHKA) and the government of West Papua for sponsoring our investigations of *Hemiscyllium* around the Bird's Head Peninsula, and are extremely grateful for the hospitality and generosity of the following dive resort and live aboard boat owners–managers and their hardworking and efficient staff for their assistance in sample collection: Max Ammer (Papua Diving, Raja Ampat Islands), Craig Howson (*True North* live aboard), Rob Sinke (Weda Bay Resort, Halmahera), Dick Knight (Loloata Resort, Papua New Guinea), Ken and Josephine Wiedenhoeft (*Putiraja* live aboard), Alberto Reija and Tony Rhodes (*Damai* live aboard), Rob Vanderloos (*Chertan* live aboard) and freelance guide Graham Abbott. Many thanks to Jürgen Pollerspöck and the Shark References database for assistance with fossil information.

References

Adolfssen, J. S., and Ward, D. J. (2014). Crossing the boundary: an elasmobranch fauna from Stevns Klint, Denmark. *Palaeontology* **57**(3), 591–629. doi:10.1111/PALA.12079

Allen, G. R., and Dudgeon, C. L. (2010). *Hemiscyllium michaeli*, a new species of bamboo shark (Hemiscyllidae) from Papua New Guinea. *Aqua – International Journal of Ichthyology* **16**(1), 19–30.

Allen, G. R., and Erdmann, M. V. (2008). Two new species of bamboo sharks (Orectolobiformes: Hemiscyllidae) from western New Guinea. *Aqua – International Journal of Ichthyology* **13**(3), 93–108.

Allen, G. R., Erdmann, M. V., and Dudgeon, C. L. (2013). *Hemiscyllium halmahera*, a new species of bamboo shark (Hemicyllidae) from Indonesia. *Aqua – International Journal of Ichthyology* **19**(3), 123–136.

Allen, G. R., Hadiaty, R. H., Unmack, P. J., and Erdmann, M. V. (2015). Rainbowfishes (Melanotaeniidae) of the Aru Islands, Indonesia with

descriptions of five new species and redescription of *M. patoti* Weber and *M. senckenbergianus* Weber. *Aqua – International Journal of Ichthyology* **21**(2), 66–108.

Allen, G. R., Erdmann, M. V., White, W. T., Fahmi, , and Dudgeon, C. L. (2016). Review of the bamboo shark genus *Hemiscyllidae* (Orectolobiformes: Hemiscyllidae). *Journal of the Ocean Science Foundation* **23**, 51–97.

Barber, P. H. (2009). The challenge of understanding the Coral Triangle biodiversity hotspot. *Journal of Biogeography* **36**(10), 1845–1846. doi:10.1111/J.1365-2699.2009.02198.x

Barraclough, T. G., and Nee, S. (2001). Phylogenetics and speciation. *Trends in Ecology & Evolution* **16**(7), 391–399. doi:10.1016/S0169-5347(01)02161-9

Cappetta, H. (1980). Les Sélaciens du Crétacé supérieur du Liban. I. Requins. *Palaeontographica. Abteilung A, Paläozoologie, Stratigraphie* **168**(1–4), 69–148.

Carter, R. M., and Johnson, D. P. (1986). Sea-level controls on the post-glacial development of the Great Barrier Reef, Queensland. *Marine Geology* **71**, 137–164. doi:10.1016/0025-3227(86)90036-8

Compagno, L. J. V. (Ed.) (2001). 'Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Volume 2. Bullhead, Mackerel and Carpet Sharks (Heterodontiformes, Lamniformes and Orectolobiformes).' (FAO: Rome, Italy.)

Compagno, L. J. V., Dando, M., and Fowler, S. (2005). 'A Field Guide to the Sharks of the World.' (Harper Collins Publishers: London, UK)

Coyne, J. A., and Orr, H. A. (2004). 'Speciation.' (Sinauer: Sunderland, MA, USA.)

de Jong, R. (1998). Halmahera and Seram: different histories, but similar butterfly faunas. In 'Biogeography and Geological Evolution of SE Asia'. (Eds R. Hall and J. D. Holloway.) pp. 315–325. (Backhuys Publishers: Leiden, Netherlands)

Dingerkus, G., and DeFino, T. C. (1983). A revision of the Orectolobiform shark family Hemscyllidae (Chondricthyes, Selachii). *Bulletin of the American Museum of Natural History* **176**, 1–93.

Drummond, A. J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214. doi:10.1186/1471-2148-7-214

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973. doi:10.1093/MOLBEV/MSS075

Fisher, S., Barry, A., Abreu, J., Minie, B., Nolan, J., Delorey, T. M., Young, G., Fennell, T. J., Allen, A., Ambrogio, L., Berlin, A. M., Blumenstiel, B., Cibulskis, K., Friedrich, D., Johnson, R., Juhn, F., Reilly, B., Shammas, R., Stalker, J., Skykes, S. M., Thompson, J., Walsh, J., Zimmer, A., Zwirko, Z., Gabriel, S., Nicol, R., and Nusbaum, C. (2011). A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. *Genome Biology* **12**, R1. doi:10.1186/GB-2011-12-1-r1

Hall, R. (1998). The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In 'Biogeography and Geological Evolution of SE Asia'. (Eds R. Hall and J. D. Holloway.) pp. 99–131. (Backhuys Publishers: Leiden, Netherlands)

Hall, R. (2002). Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences* **20**, 353–431. doi:10.1016/S1367-9120(01)00069-4

Hall, R. (2009). Southeast Asia's changing palaeogeography. *Blumea* **54**, 148–161. doi:10.3767/000651909X475941

Heist, E. J. (2004). Genetics of sharks, skates and rays. In 'Biology of Sharks and their Relatives', 2nd edn. (Eds J. C. Carrier, J. A. Musick, and M. R. Heithaus.) pp. 487–504. (CRC Press: Boca Raton, FL, USA.)

Hill, K. C., and Hall, R. (2003). Mesozoic–Cenozoic evolution of Australia's New Guinea margin in a west Pacific context. *Geological Society of Australia Special Paper* **372**, 265–290. doi:10.1130/0-8137-2372-8.265

Irestedt, M., Jønsson, K. A., Fjeldså, J., Christidis, L., and Ericson, P. G. P. (2009). An unexpectedly long history of sexual selection in birds-of-paradise. *BMC Evolutionary Biology* **9**(1), 235–246. doi:10.1186/1471-2148-9-235

Kriwet, J., and Benton, M. J. (2004). Neoselachian (Chondricthyes, Elasmobranchii) diversity across the Cretaceous–Tertiary boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology* **214**, 181–194. doi:10.1016/S0031-0182(04)00420-1

Last, P. R., and Stevens, J. D. (2009). 'Sharks and Rays of Australia.' (CSIRO Publishing: Melbourne, Vic., Australia)

Li, C., Hofreiter, M., Straube, N., Corrigan, S., and Naylor, G. J. P. (2013). Capturing protein-coding genes across highly divergent species. *BioTechniques* **54**, 321–326. doi:10.2144/000114039

Lukoschek, V., Waycott, M., and Marsh, H. (2007). Phylogeography of the olive sea snake, *Aipysurus laevis* (Hydrophiinae) indicates Pleistocene range expansion around northern Australia but low contemporary gene flow. *Molecular Ecology* **16**, 3406–3422. doi:10.1111/J.1365-294X.2007.03392.x

Mayr, E. (1944). Wallace's line in the light of recent zoogeographic studies. *The Quarterly Review of Biology* **19**(1), 1–14. doi:10.1086/394684

Meyer, M., and Kircher, M. (2010). Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols* **2010**(6), pdb.prot5448. doi:10.1101/PDB.PROT5448

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 'Proceedings of the 2010 Gateway Computing Environments Workshop (GCE)', 14 November 2010, New Orleans, LA, USA. INSPEC Accession Number 11705685, pp. 1–8. (Institute of Electrical and Electronics Engineers: Piscataway, NJ, USA)

Nosil, P., Harmon, L. J., and Seehausen, O. (2009). Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution* **24**(3), 145–156. doi:10.1016/J.TREE.2008.10.011

Pandolfi, J. M. (1993). A review of the tectonic history of New Guinea and its significance for marine biogeography. In '7th International Coral Reef Symposium', 22–27 June 1992, Guam. (Ed. R. H. Richmond.) pp. 718–728. (University of Guam Press: Guam.)

Pigram, C. J., and Davies, H. L. (1987). Terranes and the accretion history of the New Guinea orogen. *BMR Journal of Australian Geology and Geophysics* **10**, 193–211.

Polhemus, D. A., and Polhemus, J. T. (1998). Assembling New Guinea: 40 million years of island arc accretion as indicated by the distributions of aquatic *Heteroptera* (Insecta). In 'Biogeography and Geological Evolution of SE Asia'. (Eds R. Hall and J. D. Holloway.) pp. 327–340. (Backhuys Publishers: Leiden, Netherlands)

Pollerspöck, J., and Beaury, B. (2014). *Parasquatina zitteli* nov. sp. (Elasmobranchii: Orectolobiformes) aus dem Maastricht von Oberbayern (Gerhartsreiter Schichten, Siegsdorf) und Bemerkungen zur Verbreitung der Ordnung Orectolobiforme. *Zitteliana A* **54**, 147–164.

Renema, W., Bellwood, D. R., Braga, J. C., Bromfield, K., Hall, R., Johnson, K. G., Lunt, P., Meyer, C. P., McMonagle, L. B., Morrley, R. J., O'Dea, A., Todd, J. A., Wesselingh, F. P., Wilson, M. E. J., and Pandolfi, J. M. (2008). Hopping hotspots: global shifts in marine biodiversity. *Science* **321**(5889), 654–657. doi:10.1126/SCIENCE.1155674

Rosser, N. L. (2016). Demographic history and asynchronous spawning shape genetic differentiation among populations of the hard coral *Acropora tenuis* in Western Australia. *Molecular Phylogenetics and Evolution* **98**, 89–96. doi:10.1016/J.YMPEV.2016.02.004

Siverson, M. (1997). Sharks from the mid-Cretaceous Gearle Siltstone, southern Carnarvon Basin, Western Australia. *Journal of Vertebrate Paleontology* **17**(3), 453–465. doi:10.1080/02724634.1997.10010995

Skeels, A., and Cardillo, M. (2019). Reconstructing the geography of speciation from contemporary biodiversity data. *American Naturalist* **193**(2), 240–255. doi:10.1086/701125

Swofford, D. L. (2002). 'PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods)', v4.0a148 edn. (Sinauer Associates: Sunderland, MA, USA.)

Turelli, M., Barton, N. H., and Coyne, J. A. (2001). Theory and speciation. *Trends in Ecology & Evolution* **16**(7), 330–343. doi:10.1016/S0169-5347(01)02177-2

van Oppen, M. J. H., Peplow, L. M., Kininmonth, S., and Berkelmans, R. (2011). Historical and contemporary factors shape the population genetic structure of the broadcast spawning coral, *Acropora millepora*, on the Great Barrier Reef. *Molecular Ecology* **20**, 4899–4914. doi:10.1111/J.1365-294X.2011.05328.x

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27**(5), 1153–1167. doi:10.1046/J.1365-2699.2000.00489.X

Webster, J. M., Braga, J. C., Humblet, M., Potts, D. C., Iryu, Y., Yokoyama, Y., Fujita, K., Bourillot, R., Esat, T. M., Fallon, S., Thompson, W. G., Thomas, A. L., Kan, H., McGregor, H. V., Hinestrosa, G., Obrochta, S. P., and Lougheed, B. C. (2018). Response of the Great Barrier Reef to sea-level and environmental changes over the past 30 000 years. *Nature Geoscience* **11**(6), 426–432. doi:10.1038/s41561-018-0127-3

Weigmann, S. (2016). Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology* **88**(3), 837–1037. doi:10.1111/JFB.12874

Wilson, B. (2013). Patterns of life and the processes that produce them. In 'The Biogeography of the Australian North West Shelf: Environmental Change and Life's Response'. (Ed. B. Wilson.) pp. 267–369. (Elsevier: Amsterdam, Netherlands.)

Wise, G., Mulvey, J. M., and Renshaw, G. M. C. (1998). Hypoxia tolerance in the epaulette shark (*Hemiscyllium ocellatum*). *Journal of Zoology* **218**, 1–5.

Wörheide, G., Hooper, J. N. A., and Degnan, B. M. (2002). Phylogeography of western Pacific *Leucetta* '*chagosensis*' (Porifera: Calcarea) from ribosomal DNA sequences: implications for population history and conservation of the Great Barrier Reef World Heritage Area (Australia). *Molecular Ecology* **11**, 1753–1768. doi:10.1046/J.1365-294X.2002.01570.X

Yokoyama, Y., Purcell, A., Lambeck, K., and Johnston, P. (2001). Shoreline reconstruction around Australia during the Last Glacial Maximum and Late Glacial Stage. *Quaternary International* **83–85**, 9–18. doi:10.1016/S1040-6182(01)00028-3

Handling Editor: Michael Kingsford